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spa Typing of *Staphylococcus aureus* as a Frontline Tool in Epidemiological Typing▽

B. Strommenger,* C. Braulke, D. Heuck, C. Schmidt, B. Pasemann, U. Nübel, and W. Witte

National Reference Centre for Staphylococci, Robert Koch Institute, Wernigerode Branch, Wernigerode, Germany

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We determined the value of *spa* typing in combination with BURP (based upon repeat pattern) grouping analysis as a frontline tool in the epidemiological typing of *Staphylococcus aureus*, based on a random collection of 1,459 clinical isolates sent to the German Reference Centre for Staphylococci within a 6-month period. The application was found to be helpful for the classification of isolates into the particular clonal lineages currently prevalent in Germany. Due to its major advantages because of the ease of interpretation and the exchangeability of the results, the use of *spa* typing greatly simplifies communication between laboratories on both the national and the international levels. Thus, it is an excellent tool for national and international surveillance of *S. aureus* as well as for analysis of the short-term local epidemiology. However, to overcome the limitations of the BURP grouping method in terms of typing accuracy and discriminatory power, the results of the default BURP grouping method must be interpreted with caution. Additional markers, like staphylococcal chromosomal cassette *mec*, lineage-specific genes, or alternative DNA polymorphisms, are indispensable. They should be selected by dependence on the clonal lineage indicated by *spa* typing and subsequent BURP analysis as well as on the basis of the particular question to be addressed.

Staphylococcus aureus is well known both as a commensal organism on the human skin and as a leading cause of human disease responsible for a variety of diseases ranging from superficial skin infections to serious infections like pneumonia, bacteremia, and endocarditis (20). The occurrence and spread of methicillin-resistant *S. aureus* (MRSA) soon after the introduction of methicillin in clinical practice finally led to the appearance of hospital-adapted multiresistant clones, which constitute a constantly growing problem as a major cause of nosocomial infections all over the world (2, 5, 42). Additionally, the appearance of MRSA in the community (community-acquired MRSA [caMRSA]) and the potential risk of its introduction into hospitals are matters of great concern (5, 16, 31, 34).

The use of efficient and accurate epidemiological typing methods is a prerequisite for monitoring and for limiting the occurrence and spread of epidemic clones within and between hospitals. Therefore, typing systems must enable the discrimination between unrelated isolates as well as the recognition of isolates belonging to the same clonal lineage in order to determine whether epidemiologically related isolates are also genetically related (36, 38). Historically, typing of *S. aureus* mostly relied on phenotypic strain characteristics (for example, susceptibility to bacteriophages or antibiotics), but over the past two decades a variety of molecular technologies have been developed. Among those technologies, SmaI macrorestriction analysis became the "gold standard" for *S. aureus* strain typing mainly because of its excellent discriminatory power, especially for analysis of the local short-term epidemiology. Thus, much effort was put into the standardization of protocols and the interpretation of macrorestriction patterns (24, 40); nevertheless, interpretation of the results is hampered by a lack of both interlaboratory reproducibility and a common nomenclature (24).

Recent developments in the sequence-based typing of *S. aureus*, like multilocus sequence typing (MLST) (7) and *spa* typing (13), represent great improvements because of their reproducibility and ease of use and the exchangeability of the results (3). Moreover, the implementation of software algorithms for the grouping of related sequence types (STs; eBURST analysis [9] and BURP [based upon repeat pattern] analysis [32]) enable the classification of isolates into particular clonal lineages.

Previous studies have shown that there is a fairly good correlation between the clonal groupings of MRSA obtained by *spa* typing and those obtained by other typing techniques (4, 17, 23, 35, 39); additionally, we and others could demonstrate that the groupings of the *spa* types obtained by BURP analysis are generally concordant with the classifications obtained by alternative methods, like MLST-eBURST analysis or SmaI macrorestriction analysis and cluster analysis (12, 30, 37). Thus, *spa* typing enables the reliable allocation of isolates to the most prevalent epidemic lineages. However, those studies were done with collections of well-selected isolates representative of the most predominant clones within a certain geographical region. Therefore, the aim of the present study was to assess the value of *spa* typing in combination with BURP grouping as a frontline tool for routine epidemiological typing for national surveillance purposes.

MATERIALS AND METHODS

Bacterial strains. During a time span of 6 months (May to October 2006) a total of 1,490 staphylococcal isolates were sent to the National Reference Centre for Staphylococci for further characterization and typing. Isolates originated from microbiology laboratories from throughout Germany and comprised infection-related isolates (nosocomial and community acquired; $n = 711$), as well as isolates not associated with infection ($n = 371$; screening isolates from colonized

* Corresponding author. Mailing address: Robert Koch Institute, Wernigerode Branch, Burgstr. 37, D-38855 Wernigerode, Germany. Phone: 0049/3943/679 260. Fax: 0049/3943/679 317. E-mail: strommengerb@rki.de.

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TABLE 1. Typeability and diversity

Organism group(s)	Typeability[a] (%)	No. of *spa* types	No. (%) of singular *spa* types	Diversity index[b] (95% CI[c])
MRSA + MSSA (n = 1,459)	99.8	221	121 (54.8)	0.888 (0.877–0.900)
MRSA (n = 1,176)	99.8	121	60 (49.6)	0.835 (0.820–0.851)
MSSA (n = 283)	99.7	128	85 (66.4)	0.971 (0.960–0.982)

[a] According to Struelens (38).
[b] According to Hunter and Gaston (15).
[c] CI, confidence interval.

patients and personnel) or isolates for which no further information concerning the patient's medical history was available (n = 412). All isolates were cultured on sheep blood agar and were confirmed to be *S. aureus* by colony morphology and a positive plasma coagulase reaction. They were subjected to susceptibility testing by the broth microdilution method, according to the DIN 58940 method (6). Reference isolates for the current most prevalent clonal lineages in Germany and Central Europe were the following: 03-02773 (t175, ST001), 02-02424 (t002, ST005), 05-02040 (t008, ST008), 96-01678 (t032, ST022), 03-01265 (t021, ST030), 98-01907 (t018, ST036), 93-01150 (t004, ST045), 02-02404 (t044, ST080), 02-02878 (t285, ST121), 04-02981 (t003, ST225), 98-01155-2 (t001, ST228), 04-02080 (t037, ST239), 98-00406 (t051, ST247), and 98-01442 (t009, ST254). All of the reference isolates were characterized in a recent study (37).

DNA extraction. Genomic DNA was isolated from 2 ml overnight culture with a DNeasy tissue kit (Qiagen, Hilden, Germany), with lysostaphin (100 mg/liter; Sigma, Taufkirchen, Germany) used to achieve bacterial lysis.

***spa* typing and BURP analysis.** The polymorphic X region of the protein A gene (*spa*) was amplified from all *S. aureus* isolates by using the primers spa-1113f (5′-TAA AGA CGA TCC TTC GGT GAG C-3′) and spa-1514r (5′-CAG CAG TAG TGC CGT TTG CTT-3′). All sequencing reactions were carried out with an ABI Prism BigDye Terminator cycle sequencing ready reaction kit (Applied Biosystems, Foster City, CA). *Spa* types were assigned by using StaphType software (version 1.4; Ridom GmbH, Würzburg, Germany), as described by Harmsen et al. (13). By application of the BURP algorithm implemented by the software, *spa* types with more than five repeats were clustered into different groups, with the calculated cost between members of a group being less than or equal to 6.

Evaluation of *spa* typing results. Calculation of the typeability, diversity, and concordance of the *spa* typing method with the results of alternative typing methods was implemented in Ridom StaphType software (version 1.4). Phylogenetic and molecular evolutionary analyses were conducted with MEGA software (version 3.1; http://www.megasoftware.net).

Typeability. The proportion of strains that could be assigned a *spa* type was calculated as described by Struelens (38).

Diversity. The index of diversity, defined as the average probability that the typing system will assign a different type to two unrelated strains randomly sampled from a microbial population, was calculated (15). It depends on the number of types and on the homogeneity of the distribution of the strains into types. Confidence intervals for discriminatory indices were calculated as described previously (10).

Concordance. The agreement between two strain typing tests was calculated as described by Robinson et al. (28).

SmaI macrorestriction and cluster analyses. For a subset of isolates exhibiting new or uncommon *spa* types, as well as for all isolates related to *spa* type t032 (ST22), SmaI macrorestriction analysis was conducted according to the HARMONY protocol. The resulting gel images were analyzed with the BioNumerics software package (Applied Maths, Sint-Martens-Latem, Belgium) by using the Dice coefficient and were visualized as a dendrogram obtained by the unweighted pair group method with arithmetic averages, with 1% tolerance and 1% optimization settings. A similarity cutoff of 70% was used to define a cluster.

MLST and eBURST analysis. MLST was conducted as described previously (7) with a subset of isolates with new or uncommon *spa* types. The allele types and the resulting STs were assigned at the *S. aureus* MLST database via the Internet (www.mlst.net). Sequence types were clustered into groups by eBURST analysis by employing the relaxed group definition with five of seven loci (i.e., the members of a group differ at a single locus or two loci).

SCC*mec* typing. Typing of the staphylococcal chromosomal cassette *mec* (SCC*mec*) was done as described previously (43).

TABLE 2. The 10 most frequent *spa* types within MRSA and MSSA isolates

Organism group	Most frequent *spa* type[a]	No. (%) of isolates	CC or ST[b]
MRSA	t032	355 (30.2)	CC22
	t003	298 (25.3)	CC5
	t002	55 (4.7)	CC5
	t008	53 (4.5)	CC8
	t001	52 (4.4)	CC5
	t004	47 (4.0)	CC45
	t041	22 (1.9)	CC5
	t044	21 (1.8)	ST80
	t030	15 (1.3)	ST239
	t022	10 (0.9)	CC22
MSSA	**t008**	41 (14.5)	CC8
	t091	14 (4.9)	CC7
	t078	8 (2.8)	CC25
	t084	8 (2.8)	CC15
	t005	7 (2.5)	CC22
	t015	7 (2.5)	CC45
	t159	7 (2.5)	CC121
	t230	6 (2.1)	CC45
	t002	5 (1.8)	CC5
	t056	5 (1.8)	CC101

[a] Types in boldface indicate their occurrence in both MRSA and MSSA strains.
[b] Clonal lineages (as defined by MLST and eBURST analysis) are inferred from previous *spa*-MLST mappings.

RESULTS

Statistics. Among the 1,459 *S. aureus* isolates sent to the German reference center for staphylococci, 1,176 (81%) were MRSA and 283 (19%) were methicillin-susceptible *S. aureus* (MSSA).

Typeability. Among the 1,459 *S. aureus* isolates, all but 3 were typeable by *spa* typing. The nontypeable isolates comprised two MRSA isolates and one MSSA isolate. The resulting typeability figures for both MRSA isolates and MSSA isolates are summarized in Table 1.

Reproducibility. To control the intralaboratory reproducibility of the sequence-based typing method, every 50th isolate (n = 29) in this study was typed repeatedly. All previous typing results could be confirmed, thus leading to an intralaboratory reproducibility of 100%.

Diversity of *spa* types. Overall we identified 221 different *spa* types among the 1,459 *S. aureus* isolates (Table 1). However, although we analyzed only 283 MSSA isolates, we found even more different *spa* types within this population compared to the number of types detected in the much larger population of MRSA isolates. Only 28 *spa* types occurred in both populations. Additionally, a much higher percentage of *spa* types occurred only once in the MSSA population. These facts contribute to the calculation of a distinctly higher diversity index for the MSSA population (Table 1). This difference in diversity is also reflected in the proportion of isolates represented by the 10 most common *spa* types within the two populations (Table 2). These isolates constituted 38.2% of all MSSA isolates but 79% of all MRSA isolates in this study. The two predominant *spa* types among the MRSA isolates (types t032 and t003) represented more than 50% of all MRSA isolates but occurred only rarely among the MSSA isolates. The remaining eight *spa* types were distributed more homogeneously, but with two exceptions (types t008 and t002) they were also restricted mainly

isolate	*spa*-type	resistance	MLST	SCC*mec*	CC	PFGE[a]	BURP group
06-01198	t321	MSSA	ST001			A	
06-01829	t1491	MSSA	ST001		CC001		
06-01976	t1775	MSSA	ST001				
06-02086	**t091**	**MSSA**	**ST007**		**Sg #9**		
06-01111	t127	MSSA	ST852		CC001	B	
06-01420	**t359**	**MRSA**	**ST097**		**Sg #14**		
06-01042	t189	MSSA	ST188		CC001	D	
06-01626-1	t657	MSSA	ST772			E	
06-01603	**t084**	**MSSA**	**ST015**			G	**A**
06-02066	**t346**	**MSSA**	**ST015**				
06-02087	**t094**	**MSSA**	**ST015**				
06-02096	**t499**	**MSSA**	**ST015**		**Sg #10**		
06-02111	**t1687**	**MSSA**	**ST015**				
06-02114	**t085**	**MSSA**	**ST015**			H	
06-01035	t1383	MRSA	ST001		CC001	K	
02-02404	***t044***	***MRSA***	***ST080***	***IV***		Q	
06-01331	**t434**	**MRSA**	**ST080**		***Sg #8***		
06-01407	**t131**	**MRSA**	**ST080**				
06-01360	t688	MSSA	ST005			A	
06-01474	t892	MRSA	ST228			Ba	
02-02424	*t002*	*MRSA*	*ST005*	*II*		E	
04-02981	*t003*	*MRSA*	*ST225*	*II*			
06-01124	t1107	MRSA	ST225				
06-01358	t439	MSSA	ST005		CC005		**B**
06-01290	t179	MSSA	ST005				
06-01713	t447	MRSA	ST005			F	
06-01624	t001	MRSA	ST005	I		L	
06-01508	t105	MRSA	ST005			O	
98-01155-2	*t001*	*MRSA*	*ST228*	*I*		*P*	
06-01048	t608	MRSA	ST022			Aa	
06-01066	t294	MSSA	ST022			W	
06-01067	t020	MRSA	ST022				
06-01129	t379	MRSA	ST022				
06-01258	t474	MRSA	ST022		CC022		**C**
06-01737	t1015	MRSA	ST022				
06-01980	t1566	MRSA	ST022				
06-02400	t1214	MRSA	ST022				
96-01678	*t032*	*MRSA*	*ST022*	*IV*			
06-01019	t1384	MRSA	ST045			S	
06-01089	t553	MRSA	ST045				
06-01105	t040	MRSA	ST045				
06-01156	t1460	MSSA	ST045				
06-01304	t277	MRSA	SLV45	IV	CC045		**D**
06-01483	t102	MRSA	ST045				
06-01825	t015	MSSA	ST045				
06-02164	t691	MRSA	ST045				
93-01150	*t004*	*MRSA*	*ST045*	*IV*			

isolate	*spa*-type	resistance	MLST	SCC*mec*	CC	PFGE[a]	BURP group
04-02080	***t037***	***MRSA***	***ST239***	***III***	**CC008**	**M**	
06-01537-1	**t363**	**MRSA**	**ST241**				
06-01033	**t011**	**MRSA**	**ST398**		**Sg #15**	**NT**	
06-01162	**t034**	**MRSA**	**ST398**				
03-01265	*t021*	*MRSA*	*ST030*	*IV*	CC30	T	**E**
06-01300	t1487	MSSA	ST030				
06-01318	t1501	MSSA	ST030				
06-02429	t021	MRSA	SLV30	new			
98-01907	*t018*	*MRSA*	*ST036*	*II*			
06-02441	t318	MRSA	SLV30	IV		U	
06-02439	t1029	MRSA	ST008		CC008	A	
05-02040	*t008*	*MRSA*	*ST008*	*IV*		J	
06-01514	t068	MRSA	ST008				
06-01855	t190	MRSA	ST008				**F**
98-01442	*t009*	*MRSA*	*ST254*	*IV*		*K*	
98-00406	*t051*	*MRSA*	*ST247*	*I*		*L*	
06-01057	**t305**	**MRSA**	**ST617**	**IV**	**CC045**	**X**	
06-01314	t623	MSSA	ST101		CC101	A	
06-01582	t056	MSSA	ST101				
06-01871	t1541	MSSA	SLV101				**G**
06-01102	**t1439**	**MSSA**	**ST025**		**CC025**	**R**	
06-01322	**t078**	**MSSA**	**ST025**				
02-02878	*t285*	*MSSA*	*ST121*		CC121	Y	**H**
06-01807	t171	MSSA	ST123				
06-01761	**t1707**	**MSSA**	**ST101**		**CC101**	**A**	
06-01203	**t307**	**MRSA**	**ST902**	**I**	**CC022**	**Aa**	
06-01468	t1518	MSSA	ST856		Sg #13	Aa	
03-02773	***t175***	***MRSA***	***ST001***	***IV***	***CC001***	***B***	
06-01345	t1506	MSSA	new		Sg #6	C	
06-01480	t156	MSSA	ST901		Sg #7	C	
06-02002	t493	MSSA	ST182		Sg #11	Ca	
06-01872	t164	MSSA	ST020		Sg #5	F	**singletons**
06-01240	t172	MRSA	ST375	V	Sg #12	I	
06-01956	t100	MSSA	ST027		Sg #2	I	
06-01091	t1442	MSSA	ST718		Sg #1	N	
06-02026	**t136**	**MSSA**	**ST034**		**CC030**	**T**	
06-01977	t186	MSSA	ST088		Sg #3	V	
06-01583	**t1612**	**MRSA**	**SLV22**	**IV**	**CC022**	**W**	
06-02234	t1938	MSSA	new		Sg #4	Z	
06-01550	t362	MRSA	ST045		CC045	S	**excluded**

FIG. 1. Summary of typing results for a selection of 85 isolates, including 14 reference strains (indicated by italics), ordered by the results of BURP grouping. [a], Groups were defined as described in Material and Methods; Sg, singleton. The degree of typing concordance between different methods in each BURP cluster is color coded: light gray indicates excellent concordance between methods, medium gray indicates moderate concordance, and dark gray represents BURP groups with a high number of falsely classified isolates. Incorrect classifications are demonstrated in boldface type. Underlined characters represent isolates with ambiguous *spa* types.

to MRSA isolates. For the MSSA isolates, the distribution of the 10 dominant types was obviously more homogeneous, with type t008 being the only predominant clone. Apart from the two exceptions mentioned above, most *spa* types were associated with MSSA isolates.

Assignment of isolates to clonal lineages and typing concordance. The MRSA isolates as well as the MSSA isolates with the most common *spa* types found in this study could unambiguously be mapped to the corresponding clonal lineages defined by MLST as the reference method (Table 2). This was enabled predominantly by recent studies of "*spa*-MLST mapping" (the results of both *spa* typing and MLST), which are summarized at http://spa.ridom.de/mlst.shtml. For the large proportion of the uncommon *spa* types in our collection, we used the BURP algorithm to group them into definite clonal lineages on the basis of their relatedness to the *spa* types representative of each lineage. To prove the reliability of this approach, we selected 73 isolates (38 MSSA isolates and 35 MRSA isolates) with less frequent *spa* types and added 14 reference strains representative of the most predominant clonal lineages in Germany and Central Europe. These isolates were additionally typed by SmaI macrorestriction analysis and MLST as the reference methods. Subsequently, isolates were grouped by using the BURP algorithm, cluster analysis, and eBURST analysis, respectively. The resulting concordance values between the various methods were as follows: *spa* typing and MLST, 0.963; *spa* typing-BURP analysis and MLST-eBURST analysis, 0.937, and *spa* typing-BURP analysis and pulsed-field gel electrophoresis (PFGE)–cluster analysis, 0.915 (two ST398 strains were not typeable by SmaI macrorestriction analysis). The results of a more detailed analysis are provided in Fig. 1, which includes all typing results for the nine different groups generated by BURP analysis with the collection of 87 isolates. Figure 1 also visualizes the appearance of "group violations" in BURP groups A, E, F, and G. These groups contain isolates of two to five unrelated clonal complexes. To elucidate apparent "group violations" (BURP groups A, E, F, and G) in more detail, a neighbor-joining tree based on the distance matrix produced by the BURP algorithm was generated (Fig. 2). The resulting phylogenetic tree clearly demonstrates one reason for group violations: it contains deep branches (BURP groups B, C, D, E, and F) clearly separated from the others, but it also contains poorly separated ones (BURP groups A, E, and H) with rather short distances be-

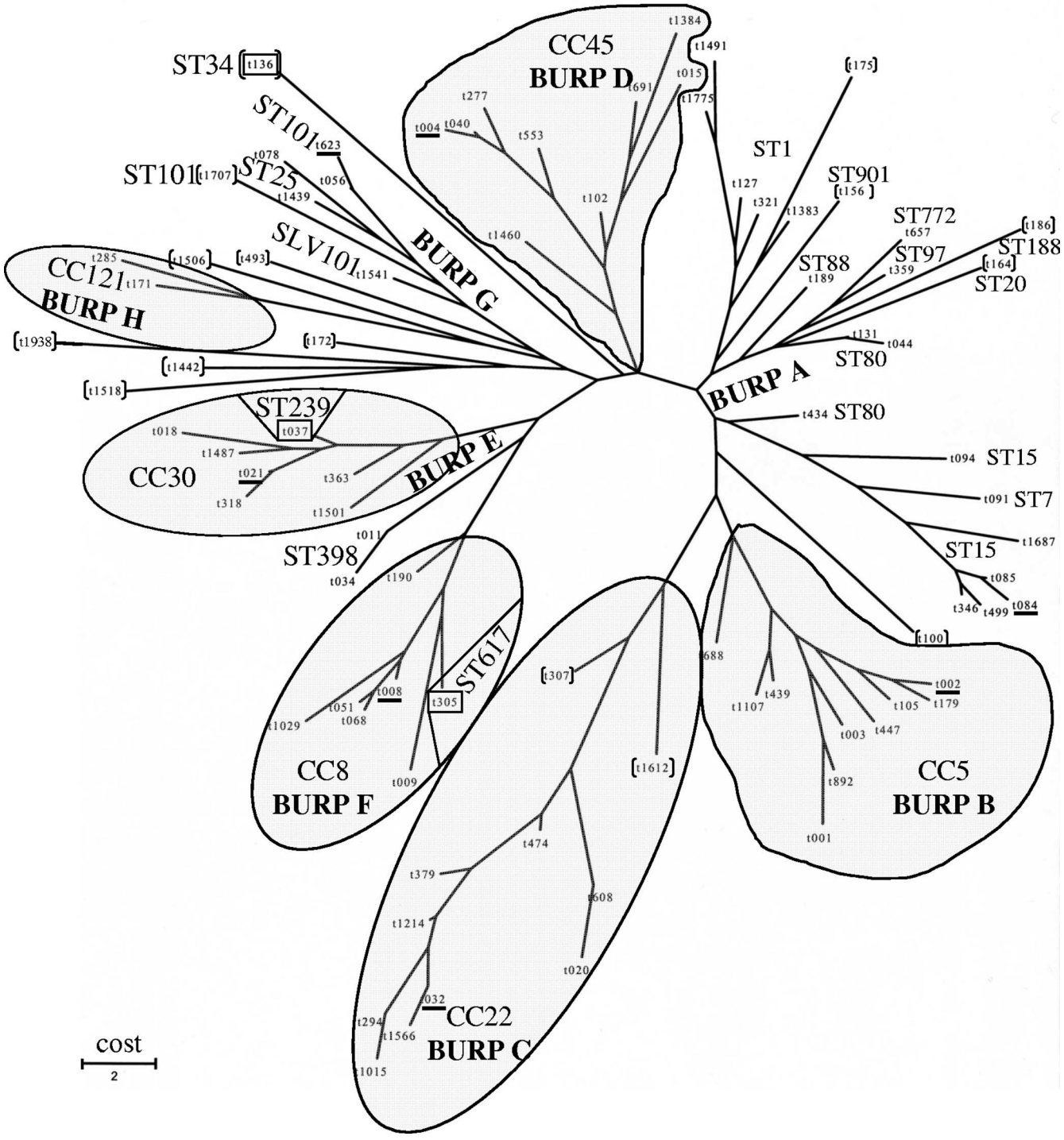

FIG. 2. Neighbor-joining tree based on the distance matrix produced by StaphType software. BURP groups as well as MLST CCs are indicated. Underlined *spa* types represent putative ancestors within the respective BURP group, as defined by use of the BURP algorithm. *Spa* types in parentheses were not assigned to a particular BURP group (singletons).

tween *spa* types within a branch as well as between neighboring branches, indicating the lack of reliability of the group designations within these branches.

Variability within a single clonal lineage. We selected one of the clonal lineages currently most frequent in Germany (Barnim MRSA [44], clonal complex 22 [CC22], as determined by MLST) for further analysis of the *spa* type variability within a single clonal lineage. We found that 37 different *spa* types were related to the CC22 reference strains, as reflected by BURP analysis. However, the SmaI macrorestriction patterns were rather homogeneous, with a high percentage of isolates being identical or obviously very closely related (Fig. 3). Clus-

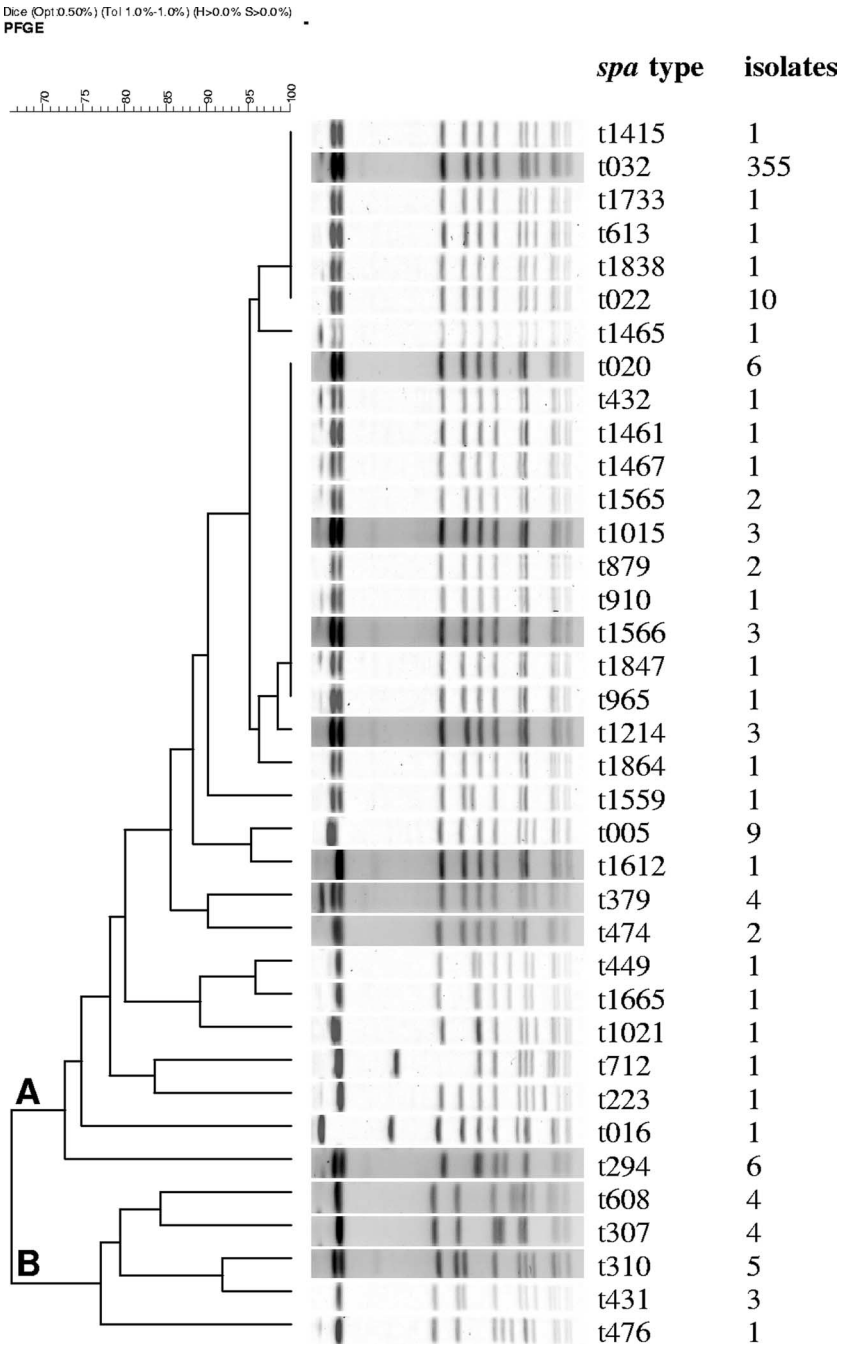

FIG. 3. SmaI macrorestriction analysis for all isolates with *spa* types belonging to MLST CC 22, as inferred from BURP analysis. The numbers represent the number of isolates exhibiting the particular *spa* types among all isolates investigated in this study.

ter analysis revealed one large cluster (Fig. 3A) and a second small cluster containing more distantly related isolates (Fig. 3B).

Epidemiological investigations. We checked isolates originating from the same hospitals for putative epidemiological relationships on the basis of their *spa* types as well as on the basis of additional medical information. The respective isolates were additionally typed by SmaI macrorestriction analysis to confirm their epidemiological relatedness. Examples of local clusters of infections with a particular clone indicating nosocomial transmission are given in Fig. 4 for two hospitals; those examples also demonstrate the stability of the *spa* types over a certain period of time, which is essential for outbreak investigations.

DISCUSSION

The aim of the present study was evaluation of the use of *spa* typing (together with BURP analysis) as a frontline tool for the routine typing of staphylococcal isolates at the German Ref-

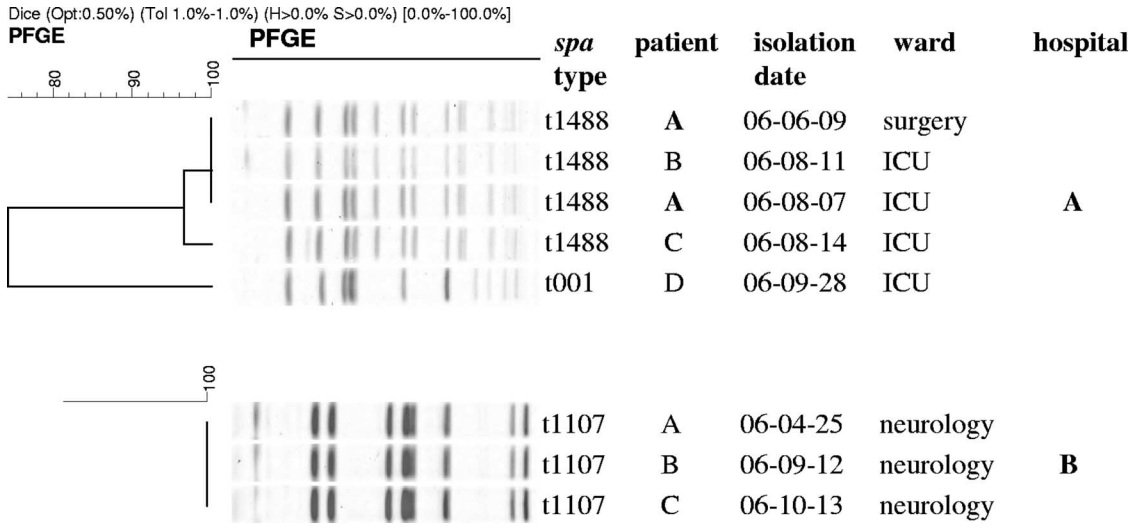

FIG. 4. Epidemiological investigation of clusters of infection. The genetic relatedness of the isolates is demonstrated by the *spa* type as well as by SmaI macrorestriction analysis. In hospital A, four identical isolates and one additional isolate were detected. In hospital B, all isolates were identical, as demonstrated by both methods applied. ICU, intensive care unit.

erence Centre for Staphylococci, in accordance with previously proposed guidelines (38) and on the basis of 6 months of experience with routine *spa* typing.

In agreement with the findings of previous studies, we found that *spa* typing has a high degree of typeability as well as excellent reproducibility in our laboratory (1). The unambiguous nomenclature also facilitates the submission of comparable typing information to international networks (for example, SeqNet [www.seqnet.org]), which is in clear contrast to methods based on fragment patterns, like PFGE, where international typing networks are often hampered by a lack of interlaboratory reproducibility as well as a common nomenclature (24). Thus, *spa* typing can be an excellent tool for the international multicenter surveillance of MRSA strains (4).

The calculation of *spa* type diversity revealed great differences between the MRSA and the MSSA isolates investigated in this study. Ideally, the calculation of diversity indices (as well as concordance values) should be done with genetically unrelated strains (38). Since this was definitely not the case in the present study, the absolute figures should be treated with caution; however, the increased diversity within the population of MSSA isolates is in concordance with the findings of other studies (8, 11). Moreover, the majority of the *spa* types within the MRSA population could unambiguously be assigned to a limited number of clonal lineages known to be prevalent in Central European countries at present (37); in contrast, within the more heterogeneous MSSA population, the *spa* types were distributed along a wider range of different clonal lineages, with more or less "MSSA-specific" lineages, like CC25, CC7, CC15, CC101, and CC121, prevailing. Only a limited number of *spa* types occurred in both the MRSA and the MSSA groups of isolates; and they were predominantly classified into the well-known MRSA lineages CC5, CC8, CC22, CC45, CC30, and ST1. These data are concordant with those from recent studies supporting the view that the currently predominating clonal lineages of MRSA arose from a few locally and globally successful MSSA clones by the acquisition of SCC*mec* on multiple occasions (8, 11, 26). However, due to a lack of studies with larger MSSA population structures, statements about the times and the places of appearance of MRSA strains can be only speculative. Thus, larger parallel genetic studies with local MSSA and MRSA populations are necessary to understand the interactions of the two populations.

The typing data resulting from those studies can also be used to enlarge our knowledge of *spa*-MLST mappings, which is extremely useful for the daily routine typing of *S. aureus*, in which the BURP algorithm together with "reference *spa* types" enables the classification of isolates into particular clonal lineages. This work, as well as previous studies, has shown that, in general, the typing concordance between *spa* typing-BURP analysis and alternative methods is high; however, the occurrence of "group violations" (12) associated with particular BURP groups and clonal lineages was also demonstrated. Some of these misclassifications (in BURP groups A, E, and G) are due to related *spa* repeat successions in isolates of different clonal lineages, possibly caused by recombination events in the *spa* locus (reflected by insufficiently branched parts of the phylogenetic tree). The highest degree of misclassification and, at the same time, the epidemiologically most relevant misclassification was found in BURP group A, which included isolates of clonal lineages CC1, ST80, ST7, ST15, and ST97 (see also reference 12). Since isolates of CC1 and ST80 represent important caMRSA clones, their unambiguous recognition is of particular importance to prevent their spread in the community and their introduction into hospitals. Isolates of ST398 (*spa* types t034 and t011 in BURP group E) constitute another clonal lineage of special interest that is falsely grouped. Isolates of this clonal lineage are increasingly isolated from animals throughout Europe and were also found in hospital patients; thus, these isolates possibly possess some zoonotic potential (45) and it is crucial that they be identified in a timely manner. To reduce this type of misclassification, Mellmann et al. (22) suggested that the parameter "costs" (costs $\leqslant$ 4) for the definition of BURP groups should be reduced, and

this was also implemented as the default setting in the last update (version 1.5) of the StaphType software. However, this can solve the problem only partly (separation of ST398 from group E and ST101 from group G) and does not lead to the unambiguous separation of ST80 and CC1 isolates (data not shown). On the other hand, it leads to further separations within other, fully concordant BURP groups (for example, group D, clonal lineage CC45) and to an increase in the numbers of nongroupable isolates. Therefore, we favor the detection of lineage-specific markers for the differentiation within BURP groups noted for group violations.

Other reasons for group violations are the previously described large chromosomal replacements, encompassing the *spa* locus. Robinson and Enright described this phenomenon for ST239 (CC8), ST241 (CC8), and ST34 (CC30) and speculated about the lineage specificity of these events (27). In addition to the previously described STs (ST239/*spa* type t037 and ST241/*spa* type t363 in BURP group E; ST34/*spa* type t136, BURP singletons) we found another isolate (06-01057, ST617/*spa* type t305, BURP group F) which is grouped along with *spa* types representative of CC8 but which was revealed to belong to CC45, as defined by eBURST analysis. Preliminary sequencing results for *sas* genes (26) revealed a similar chromosomal replacement event involving progenitors of CC8 and CC45. This indicates that a wider range of lineages than was assumed previously is involved in recombination events.

In BURP groups B and F, the grouping is almost fully concordant with the distribution of the CCs generated from MLST data by eBURST analysis (demonstrated by the deeply branched parts in the phylogenetic tree [Fig. 2]); however, a more discriminatory grouping (which is partially provided by PFGE) would be desirable, as these groups contain different clonal lineages important as hospital-adapted MRSA lineages in different geographical regions (group B, isolates of CC5 encompassing ST5, ST225, and ST228; group F, isolates of CC8 encompassing ST8, ST247, and ST254 [5]). Although in most instances a certain *spa* type can be mapped to one particular MLST, BURP group B contains one example of the ambiguity of some *spa* types; isolates 06-01624 and 98-01155-2 both exhibited *spa* type t001 but revealed ST5 and ST228, respectively. Those ambiguities might be due to the parallel evolution of strains originally belonging to the same clonal lineage. They were also detected in other *spa* types and clonal lineages (predominantly within CC5 and CC8) and are summarized at http://spa.ridom.de/mlst.shtml. The lack of discrimination following from this convergent evolution of different lineages can be compensated for in part by additional SCC*mec* typing, which enables further differentiation of the isolates within the respective clonal lineages; however, it is not able to distinguish all possible clones. Thus, additional lineage-specific markers must be employed in some instances (for example, for ST8/t008, while SCC*mec* type I was found in the "classical" hospital-acquired MRSA isolates [haMRSA], SCC*mec* type II was detected in haMRSA "Irish-1"; in contrast, SCC*mec* type IV is common in haMRSA epidemic MRSA types 2 and 6, as well as in caMRSA USA300 [5, 41]).

The successful application of *spa* typing for the detection of clusters of infections or transmission events was demonstrated in different recent studies (14, 21, 33). We were also able to monitor clusters of infections over even longer periods of time, thus also corroborating the in vivo stability of the *spa* locus as a molecular marker in epidemiological investigations. However, as soon as widespread *spa* types (especially types t032 and t003) are involved, local epidemiological investigations encounter difficulties. Although our study covers a sample of only about 10% of the MRSA isolates recovered in German hospitals during the time span of the study, it clearly indicates the existence of endemic clones (t032/ST22 and t003/ST225) prevalent in certain geographical regions. For clonal lineage CC22, which is also endemic in the United Kingdom, we could demonstrate that although it encompasses a large variety of different *spa* types, only a few of them have the ability to spread efficiently within hospitals. The same phenomenon was also demonstrated by alternative typing methods (25; W. Witte, unpublished data). The endemic spread of these highly successful types finally leads to a lack of discrimination in local hospital epidemiology. To overcome this limitation, recent studies suggest the use of a combination of different typing techniques to increase the ability to discriminate isolates (18, 29). We have previously described the use of a combination of two techniques (SmaI macrorestriction analysis and *dru* typing) for the successful subtyping of isolates of CC45 (46).

In conclusion, we demonstrated the value of *spa* typing in combination with BURP analysis as a frontline tool for routine epidemiological typing, based on a random sample of isolates. We could show that this approach yields highly reproducible and interchangeable information that may be used both for local epidemiology and for national as well as international surveillance of MRSA and MSSA lineages. However, to overcome the limitations of a single locus-based molecular typing method, the use of additional markers is indispensable. To minimize time as well as cost, those markers should be selected on the basis of the clonal lineage inferred by *spa* typing-BURP analysis as well as on the basis of the question to be addressed. Additional targets can be SCC*mec*, lineage-specific virulence or resistance genes, or alternative polymorphic regions of the *S. aureus* chromosome. When virulence or resistance genes are used, one must consider the fact that a majority of these genes reside on mobile genetic elements, which are subject to frequent exchange between different lineages (19).

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